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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant: O'Gorman and Wahl

Title: SITE-SPECIFIC RECOMBINATION IN EUKARYOTES AND CONSTRUCTS USEFUL THEREFOR

Appl. No.: 08/919,501

Filing Date: August 28, 1997

Examiner: M. Wilson

Art Unit: 1633

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TRANSMITTAL OF APPEAL BRIEF

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Sir:

Transmitted herewith is an Appeal Brief in the above-identified application.

[X] Applicant claims small entity status. See 37 CFR 1.27.

[X] Applicant hereby petitions for an extension of time under 37 C.F.R. §1.136(a) for the total number of months checked below:

Atty. Dkt. No. SALK2190
(088802-5001)

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		EXTENSION FEE TOTAL:	$920.00
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Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date: March 11, 2002

By _____

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PATENT
Attorney Docket No.: SALK2190
(088802-5001)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of: O'Gorman and Wahl	Group Art Unit: 1633
Application No.: 08/919,501	Examiner: M. Wilson
Filing Date: August 28, 1997	**CERTIFICATE OF FACSIMILE TRANSMISSION** I hereby certify that this paper is being facsimile transmitted to the United States Patent and Trademark Office, Washington, D.C., on the date below.
For: SITE-SPECIFIC RECOMBINATION IN EUKARYOTES AND CONSTRUCTS USEFUL THEREFOR	Teresa Spehar Printed Name Signature March 11, 2002 Date

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Washington, D.C. 20231

APPEAL BRIEF

Sir:

Applicant (herein, "Appellant") hereby appeals the final rejection of claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51 in the above-identified application (Office Action, Paper No. 25, mailed July 13, 2001) and submits this Appeal Brief in accordance with 37 C.F.R. § 1.192. This Appeal Brief is accompanied by the requisite fee set forth in 37 C.F.R. § 1.17(c). If this fee is incorrect or if any additional fees are due in this regard, please charge or credit Deposit Account No. 50-0872 for the appropriate amount.

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In re Application of: O'Gorman and Wahl
Application No.: 08/919,501
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PATENT
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(088802-5001)

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I. Table of Authorities and References

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Mullins and Mullins, *J. Clin. Invest.* **98**:S37-S40, 1996 13
Peschon et al., *Ann. N.Y. Acad. Sci.* **564**:186-197, 1989 16
Peschon et al., *Proc. Natl. Acad. Sci. USA* **84**:5316-5319, 1987 16
Suzuki et al. eds., An Introduction to Genetic Analysis, 4th Ed., 1989 18
Zambrowicz et al., *Proc. Natl. Acad. Sci. USA* **90**:5071-5075, 1993 16

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II. Real Party in Interest

The subject application is owned by The Salk Institute for Biological Studies of La Jolla, California by virtue of the assignment recorded February 23, 1998.

III. Related Appeals and Interferences

Appellant is not aware of any appeal or interference that may directly affect, be directly affected by, or have a bearing on the Board's decision in the pending appeal.

IV. Status of Claims

The present application, USSN 08/919,501, was filed on August 28, 1997 with original claims 1-45. Claims 11, 17 and 27 were cancelled without prejudice in the amendment filed on June 17, 1999 (Paper No. 9). Claims 1-10, 16, 25, 33 and 45 were cancelled without prejudice and claims 46-51 were newly presented in the preliminary amendment filed with a request for a continued prosecution application on September 29, 2000 (Paper No. 20). Claims were last amended by Appellant in the response filed on April 24, 2001 (Paper No. 24).

On October 10, 2001, Appellant timely filed a Notice of Appeal from the decision of the Examiner mailed July 13, 2001 (Office Action, Paper No. 25) maintaining the rejection of claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51. Accordingly, the claims involved in this appeal are claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51 (attached hereto as Appendix A).

V. Status of Amendments

No amendments have been submitted pursuant to the final Office Action mailed July 13, 2001 (Paper No. 25). The text of the complete set of claims involved in this appeal is provided as Appendix A, reflecting entry of the last amendment filed by Appellant on April 24, 2001 (Paper No. 24).

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VI. Summary of the Invention

The present invention is based on the discovery that recombinase-encoding nucleic acid constructs can be incorporated into the genome of embryonic stem (ES) cells to be later activated and expressed during the development of transgenic organisms. These constructs are expressed at high levels in the germline of transgenic organisms by means of germline-specific promoters, without being expressed to a functionally significant extent in either ES cells (i.e., in culture) or in embryonic or adult somatic tissues (see, for example, specification at page 18, lines 16-35). A schematic of the present invention is illustrated below in Figure A.

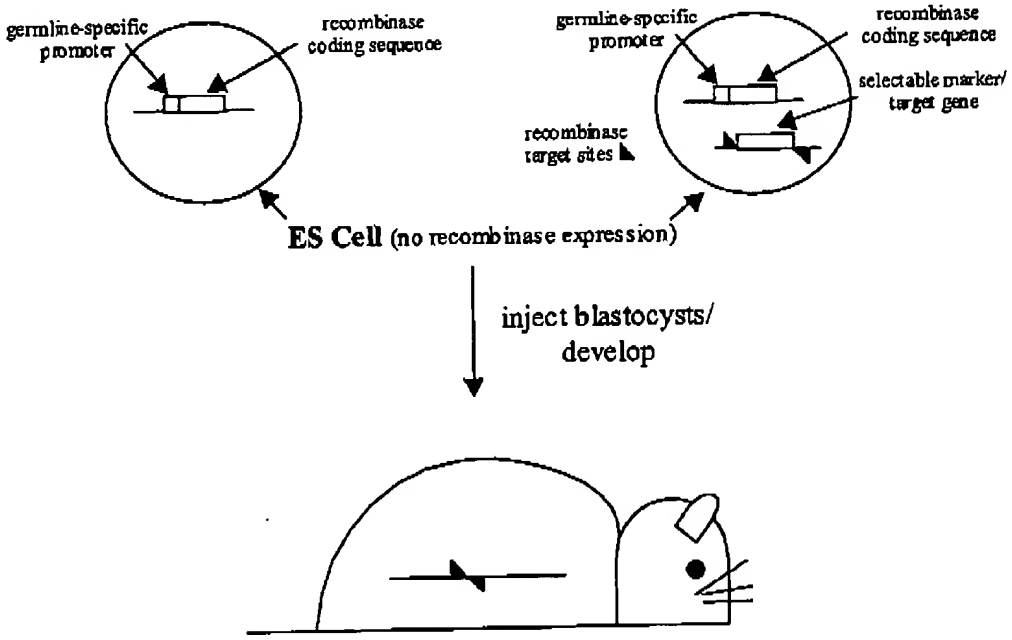

Figure A

Accordingly, the present invention provides transgenic mammalian ES cells whose genomes are transformed with recombinase-encoding nucleic acid constructs (claims 12-15, 18-24, 26 and 49-51) useful for preparation of transgenic animals, such as mice, (see, for example, specification at page 8, line 27 through page 9, line 23). The nucleic acid constructs comprise a mammalian germline-specific promoter operatively associated with a recombinase coding sequence (see, for example, specification at page 5, line 10 through page 6, line 28). The genome of such ES cells can further contain a transcriptionally active selectable marker flanked by recombinase recombination sites and/or a nucleic acid fragment flanked by recombinase recombination sites specific for a different recombinase from the recombination sites that flank the selectable marker (see, for example, specification at page 10, line 26 through page 11, line 5). Because the germline-specific promoter directs recombination events in the germline, but only to a *de minimis* amount in other tissues, embryos derived from such ES cells contain a transgenic allele, such as is caused by recombination at recombinase target site(s) (see, for example, specification at page 10, lines 16-30).

In addition, when such modified ES cells contain a nucleic acid fragment flanked by recombinase recombination sites specific for a different recombinase from the recombinase expressed in the germline (i.e., different from the recombination sites that flank the selectable marker), such as under control of an inducible or tissue specific promoter, recombination of the target site can be controlled to occur in a specific tissue (for example, somatic) or in an inducible manner (see, for example, specification at page 13, line 28 through page 14, line 3). Moreover, because the ES cells contain a germline-specific promoter operatively linked to the recombinase encoding gene, the transcriptionally active selectable marker can be excised by passage of the genome derived from such ES cells through gametogenesis (claims 28-31) (see, for example, specification at page 11, line 25 through page 12, line 5). ES cells obtained by crossing the genome of the transgenic gamete with a wild type genome can be used to obtain ES cells in which the transgene is stably incorporated into the genome, but the selectable marker is excised. Excision of the marker without excision of the allele of interest allows any observed phenotype to be more confidently ascribed to the mutation of interest rather than to some combination of that mutation and

the transcriptionally active marker (see, for example, specification at page 15, line 30 through page 16, line 12).

In accordance with the present invention, there are also provided methods for the production of transgenic animals (i.e., containing recombinant alleles; claims 32, 34-42, 44, and 46-48) (see, for example, specification at page 14, line 31 through page 15, line 8), as well as methods for the conditional assembly of functional genes for expression in eukaryotic cells by recombination of individual inactive gene segments from one or more genes of interest using invention ES cells containing recombinase responsive nucleic acids (claim 43) (see, for example, specification at page 14, lines 11-30). Thus, the present invention provides novel methods of general DNA manipulation with broad applicability to any gene of interest.

VII. Issues

1. 35 U.S.C. § 112, first paragraph Rejection of Claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51

Whether claims to non-human mammalian embryonic stem (ES) cells comprising a germline-specific promoter operatively associated with a recombinase coding sequence and methods of use thereof are enabled by the specification as filed when any mammalian ES cells can be prepared and developed using any germline-specific promoter/recombinase construct following the teachings of the specification and the knowledge of one skilled in the art.

2. 35 U.S.C. §112, second paragraph Rejection of Claims 12, 28-32, 34-44 and 46-51

Whether claims containing language that is clear to one of skill in the art in light of the specification are definite so as to particularly point out and distinctly claim the subject matter which Appellant regards as the invention.

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VIII. Grouping of Claims

Claims 12-15, 18-24 and 26 stand or fall together; claims 28-31 stand or fall together; claims 32 and 34 stand or fall together; claims 35-39 stand or fall together; claims 40-42 stand or fall together; claim 43 stands or falls alone; claim 44 stands or falls alone; claim 46 stands or falls alone; claim 47 stands or falls alone; claim 48 stands or falls alone; claim 49 stands or falls alone; claim 50 stands or falls alone; and claim 51 stands or falls alone.

Specifically, claims 12-15, 18-24, and 26 relate to non-human embryonic stem (ES) cells containing a nucleic acid construct comprising a mammalian germline-specific promoter operatively associated with a recombinase coding sequence, whereas claim 49 relates to such cells wherein the ES cell is a rodent cell, and claim 50 relates to such cells wherein the ES cell is a mouse cell, and claim 51 relates to such cells wherein the ES cell is a livestock cell. The Examiner has acknowledged enablement of "a mouse ES cell whose genome comprises a construct comprising a nucleic acid sequence encoding recombinase operative linked to the MP1 promoter" (Office Action, Paper No. 25 at page 2, lines 8-10). Thus, claims broadly directed to non-human embryonic stem cells, i.e., claims 12-15, 18-24 and 26, stand or fall together; while claims directed to each of the specific stem cells, i.e., claims 49, 50 and 51, stands or falls alone.

Claims 28-31 stand or fall together as they each relate to a method for excision of a selectable marker from ES cells comprising a first nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence and a second nucleic acid construct comprising a selectable marker flanked by two recombinase recombination target sites recognizable by the recombinase encoded by the first nucleic acid construct.

Claims 32 and 34 stand or fall together as they each relate to a method for the production of recombinant alleles in a transgenic non-human animal using ES cells comprising a first nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence and a second nucleic acid construct comprising a nucleic acid

fragment flanked by at least two recombinase recombination target sites recognizable by the recombinase encoded by the first nucleic acid construct. Claim 46 relates to such a method wherein the animal is a rodent. Claim 47 relates to such a method wherein the animal is a mouse. The Examiner has acknowledged enablement of "a method of making a transgenic mouse comprising implanting the mouse ES cells . . . into a host female such that a transgenic mouse is obtained" (Office Action, Paper No. 25 at page 2, lines 10-11). Thus, claims 32 and 34 stand or fall together; while each of claims 46 and 47 stands or falls alone.

Claims 35-39 stand or fall together as they each relate to a method for the production of recombinant alleles in a rodent using ES cells comprising a first nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence and a second nucleic acid construct comprising a nucleic acid fragment flanked by at least two recombinase recombination target sites recognizable by the recombinase encoded by the first nucleic acid construct. However, the claims differ from the previous group (claims 32 and 34) by developing the ES cells through gametogenesis without causing recombination of the recombination target sites. Claim 48 relates to such a method wherein the rodent is a mouse. Thus, claims 35-39 stand or fall together; while claim 48 stands or falls alone.

Claims 40-42 stand or fall together as they each relate to a method for the production of recombinant alleles using ES cells comprising one nucleic acid construct simultaneously comprising a germline-specific promoter operatively associated with a recombinase coding sequence, a nucleic acid fragment flanked by a first pair of recombination target sites and a selectable marker flanked by a second pair of recombination sites.

Claim 43 stands or falls alone as it relates to a method for the conditional assembly of functional gene(s) using ES cells comprising individual inactive gene segments and a nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence.

Claim 44 stands or falls alone as it relates to a method for the generation of a recombinant non-human animal using ES cells comprising a germline-specific promoter operatively associated with a recombinase coding sequence.

IX. Argument

1. 35 U.S.C. § 112, first paragraph Rejection of Claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51

The rejection of claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51 under 35 U.S.C. § 112, first paragraph, as allegedly being non-enabled is respectfully submitted to be in error for the following reasons. Indeed, this rejection should be reversed because the Examiner has failed to present any evidence or reasoning supporting the alleged non-enablement of the rejected claims. Instead, the Examiner has read an additional requirement into the claims in an attempt to meet the burden of establishing a reasonable basis to question the enablement provided by Appellant's disclosure.

a. The *prima facie* case

The legal standard for enablement is "whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation" (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988); MPEP § 2164.01(b)).

The first paragraph of 35 U.S.C. § 112 requires only objective enablement of the claims (*In re Marzocchi*, 169 USPQ 367, 369 (CCPA 1971)). Enablement may be achieved either by the use of illustrative examples or by broad terminology (*Id.*). "A specification . . . which contains a teaching of the manner . . . of making and using the invention in terms which correspond in scope to those used in" the claims satisfies the objective enablement requirement (*Id.*).

Furthermore, as long as the specification "discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim", the enablement requirement of 35 U.S.C. § 112 is satisfied (*In re Fisher*, 166 USPQ 18, 24 (CCPA 1970); MPEP § 2164.01(b)). As set forth in detail below, the scope of the rejected claims is fully commensurate with the teachings in the specification; therefore, the specification objectively enables the instant claims.

To bring the objective enablement of the claims into question, it is incumbent upon the Examiner to advance some evidence or reasoning supporting the alleged non-enablement of the rejected claims (*In re Dinh-Nguyen*, 181 USPQ 46, 47 (CCPA 1974)). The Examiner has failed to meet this burden here. The Examiner merely asserts that the specification allegedly "does not enable any non-human mammalian ES cells, any germline-specific promoter or a method of making any transgenic animal as broadly as claimed" (Office Action, Paper No. 25, at page 4, lines 16-17). Unfortunately, however, no reasoning or evidence in support of the rejection is provided. Instead, the Examiner only provides conclusory statements that the specification allegedly does not contain certain express teachings, simply because such teachings are not present in a working example. In efforts to support the failure to provide the necessary reasoning or evidence in support of the rejection, the Examiner maintains that the "rejection is based on how to determine the phenotype of animals created from such ES cells" (Office Action, Paper No. 25 at page 6, line 2). However, the present claims do not require one to determine such a phenotype.

Therefore, the Examiner's statements are not sufficient to establish a *prima facie* case of non-enablement because they do not demonstrate that the allegedly absent teachings are required to enable the skilled artisan to practice the claimed invention without an undue amount of experimentation. Because the Office Action lacks the evidence or scientific reasoning required to rebut Appellant's objective enablement, this rejection should be reversed (MPEP § 2164.04).

b. The specification reasonably provides enablement for ES cells of any non-human mammal

Appellant respectfully disagrees with the Examiner's assertion that the specification allegedly "does not reasonably provide enablement for any mammalian ES cell . . ." (see Office Action, Paper No. 25, at page 2, line 13). Appellant respectfully submits that one of skill in the art would readily be able to make and use any mammalian ES cell as claimed, following the teachings of the specification and the knowledge of the art. Indeed, the Examiner acknowledges that the specification is "enabling for a mouse ES cell . . ." (see Office Action, Paper No. 25, at page 2, lines 8).

Appellant respectfully submits that the techniques used to make and use ES cells are generally applicable to ES cells of any mammalian animals and therefore, invention methods should not be restricted to the working examples performed in mice. It is clear that mice are merely used as a model organism. The specification explicitly teaches that a variety of animal species are contemplated, including mouse, rat, rabbits, swine, ruminants, poultry, etc. (see, for example, specification at page 8, lines 30-34).

Appellant further disagrees with the Examiner's assertion that the "specification does not teach making any ES cells other than mouse ES cells . . ." (Office Action, Paper No. 25, at page 4, line 18). Contrary to the Examiner's assertion, the specification contemplates use of various mammalian ES cells (see, for example, specification at page 8, lines 30-34); and methods of making and manipulating various mammalian ES cells were known in the art at the time of filing. Indeed, the Examiner's assertion is without merit because the Examiner does not provide any evidence or scientific reasoning required to rebut Appellant's objective enablement of mammalian ES cells. Moreover, one of the Examiner's own references of record illustrates the state of the prior art, teaching that pluripotent rat, sheep and cattle ES cells capable of producing chimeric offspring have been reported (Mullins and Mullins, *J. Clin. Invest.* 98:S37-S40, 1996, at page S38, column 1, lines 6-11). Therefore, the specification as filed clearly enables one of skill in the art to make and use any mammalian ES cell as claimed.

Accordingly, the specification clearly enables one of skill in the art to make and use any mammalian ES cell according to claims 12-15, 18-24 and 26.

Claim 49 is separately patentable because it is directed to ES cells that are rodent cells, being a narrowing subset of mammalian cells. The specification clearly enables claim 49.

Claim 50 is separately patentable because it is directed to ES cells that are mouse cells, being a narrowing subset of rodent cells. The specification clearly enables claim 50, indeed, the Examiner has admitted enablement of mouse ES cells containing a nucleic acid construct as noted above.

Claim 51 is separately patentable because it is directed to ES cells that are livestock stem cells, being a different narrowing subset of mammalian cells. The specification clearly enables claim 51.

c. The specification reasonably provides enablement for any germline-specific promoter

Appellant respectfully disagrees with the Examiner's assertion that the specification allegedly "does not reasonably provide enablement for any . . . germline-specific promoter. . ." (see Office Action, Paper No. 25, at page 2, lines 13-14). Appellant respectfully submits that one of skill in the art would be able to make and use any germline-specific promoter as claimed, following the teachings of the specification and the knowledge of the art. Indeed, the Examiner acknowledges that the specification is "enabling for a mouse ES cell whose genome comprises a construct comprising a nucleic acid sequence encoding recombinase operatively linked to the MP1 promoter. . ." (see Office Action, Paper No. 25, at page 2, lines 8-10).

Appellant respectfully submits that the MP1 promoter is only an exemplary germline-specific promoter, and thus the claims should not be restricted to the working examples in light of the additional disclosure. The specification explicitly teaches a variety of such promoters, and incorporates several references which teach various germline-specific promoters (see, for example, specification at page 6, lines 1-12).

Appellant further disagrees with the Examiner assertion that the "specification has not provided adequate guidance indicating the promoters have equivalent function as MP1 in transgenic mice" (Office Action, Paper No. 25, at page 5, lines 4-6). Appellant respectfully submits that the specification provides exemplary germline-specific promoters that have equivalent function to the MP1 promoter. The MP1 promoter is the spermatid-specific promoter from the protamine 1 gene. Other germline-specific promoters contemplated include promoters from the spermatid-specific protamine 2 gene, the spermatid-specific promoter from the c-kit gene, the sperm-specific promoter from angiotensin-converting enzyme, the oocyte-specific promoters from the ZP1, ZP2 and ZP3 genes, and the like. The Examiner has failed to provide any evidence or scientific reasoning required to rebut Appellant's objective enablement of these germline-specific promoters.

Multiple germline-specific promoters are explicitly provided, with corresponding references, in the specification (see, for example, specification at page 6, lines 1-12). These promoters have been shown to be germline-specific in the art. It is therefore incumbent upon the Examiner to provide reason to doubt that they will retain such specificity when coupled to the gene being introduced. These promoters have been shown to have equivalent function to the MP1 promoter, i.e. being germline specific promoters that turn on gene expression in the target germline cells. Therefore, it is further incumbent upon the Examiner to provide reason to doubt the equivalent functionality of all such germline-specific promoters.

Furthermore, Appellant respectfully disagrees with the Examiner's assertion that the MP1 promoter is not germline-specific because it expresses background levels of protein in the heart, brain and spleen. Applicants respectfully submit that it is well known in the art that the term "tissue-specific" is indeed equivalent to the substantially exclusive expression described in the specification (see specification at page 8, lines 15-18). Background levels of expression that are expected to be substantially lower (for example, 100 fold lower at the highest) than observed in the specifically targeted cells, are not functionally significant (see, for example, specification at page 23-25, Example 4).

In support of Appellant's usage, references in the art consistently refer to spermatid-specific expression of genes containing the MP1 promoter, especially in the context of transgenic mice (Peschon et al., *Proc. Natl. Acad. Sci. USA* 84:5316-5319, 1987; Peschon et al., *Ann. N.Y. Acad. Sci.* 564:186-197, 1989; Zambrowicz et al., *Proc. Natl. Acad. Sci. USA* 90:5071-5075, 1993; an abstract each of which is enclosed herein as Appendix B). Simply, the Examiner's position cannot be reconciled with the state of the art, wherein MP1 is widely accepted by those of skill in the art to be a germline-specific promoter.

Therefore, the specification as filed clearly enables any germline-specific promoter. Accordingly, one of skill in the art would not have to undertake undue experimentation to make and use any mammalian ES cell containing any germline-specific promoter, according to claims 12-15, 18-24 and 26. As noted above, each of claims 49, 50 and 51 are separately patentable because they cover narrowing subsets of ES cells. Each of claims 49, 50 and 51 are clearly enabled by the specification as filed.

d. The specification reasonably provides enablement for methods using the ES cells

Appellant respectfully disagrees with the Examiner's assertion that the specification allegedly "does not reasonably provide enablement for . . . method of making any transgenic animal or recombinant allele as broadly claimed" (see Office Action, Paper No. 25, at page 2, lines 13-15). Appellant respectfully submits that one of skill in the art would be able make and use the invention methods as claimed, following the teachings of the specification and the knowledge of the art. Indeed, the Examiner acknowledges that the specification is "enabling for . . . a method of making a transgenic mouse comprising implanting the mouse ES cells above [whose genome comprises a construct comprising a nucleic acid sequence encoding recombinase operatively linked to the MP1 promoter] into a host female such that a transgenic mouse is obtained. . ." (see Office Action, Paper No. 25, at page 2, lines 8-11). Appellant respectfully submits that the claims should not be restricted to the working examples in light of the additional disclosure.

Appellant respectfully submits that the Examiner is improperly construing the elements of the present claims in an attempt to support the rejection. The Examiner repeatedly asserts a requirement for determining the phenotype of transgenic animals that result from the invention methods. Indeed, the Examiner states that the "rejection is based on how to determine the phenotype of animals created from such ES cells" (emphasis added, see Office Action, Paper No. 25, at page 6, lines 1-2). It is respectfully submitted that this is not a requirement of the present methods.

The present invention contemplates methods of generating an array of sophisticated mutations in mammals. For example, subtle and conditional mutations require generation of alleles with minimal structural alterations, and certain approaches will in fact generate null alleles (see specification, for example, at page 16, lines 4-12). In effect, invention methods determine a "molecular phenotype" for analysis.

Appellant respectfully disagrees with the Examiner's emphasis on phenotype because the phenotype of the animal is not a requirement of the present claims. Moreover, there is no requirement in the present methods for generating an outwardly manifesting phenotype as required by the Examiner. The Examiner does not provide any explanation or reasoning as to why the phenotype of the animal is relevant to the rejection of the claims. The phenotype of the animal is not a requirement of the present claims. The Examiner asserts that the "state of the art at the time of filing was such that the phenotype of transgenic animals was unpredictable . . ." (emphasis added, see Office Action, Paper No. 25, at page 3, lines 5-6). The Examiner continues this line of reasoning by stating that "recombinase expression may not alter the phenotype of the animal" (emphasis added, see Office Action, Paper No. 25, at page 3, lines 15-16). The present invention methods do not require nor contemplate that an outwardly manifesting phenotype of the animal be necessarily altered in any way.

The Examiner's comments concerning the level of expression required to alter the phenotype and the prediction of the resulting phenotype are simply not relevant to the present claims (see Office Action, Paper No. 25, at page 6, lines 4-6). By definition, a recombinant

allele or a transgenic animal is created upon incorporation of foreign DNA, whether or not any outward phenotype is manifested. "A eukaryotic organism that develops from a cell into which new DNA has been introduced is called a transgenic organism" (An Introduction to Genetic Analysis, 4th Ed., 1989, Suzuki et al. eds.).

Appellant respectfully submits that the invention methods create a recombinant allele or transgenic animal. The generation of a recombinant allele or transgenic organism as defined in the art could readily be monitored by either detecting expression of the introduced DNA (for example, the marker gene, or a second type of recombinase) or by using PCR analysis to detect the introduced DNA within the genomic DNA. These detection methods are described in detail in the specification (see, for example, Example 4 at pages 23-25) and are well-known in the art. In effect, a "molecular phenotype" is defined by the incorporation of foreign introduced DNA, and is detectable by the methods described herein and known in the art. It is not necessary to predict the level of recombinase expression or the phenotype of the resulting animal before performing the actual manipulations of the ES cells, since they are readily determined following introduction of the DNA of interest. Thus, the specification clearly teaches how to make and use transgenic animals.

Moreover, Appellant respectfully submits that the Examiner has misinterpreted the results of Example 3, with respect to the creation of the P2Bc mice. The Examiner correctly observes that ProCre transgenic mice were first made by transfecting mouse ES cells with a nucleic acid construct comprising a sequence encoding Cre recombinase operatively linked to the MP1 promoter (see Office Action, Paper No. 25, at page 3, lines 17-19). However, the Examiner then continues by erroneously stating that "ProCre transgenic mice were bred with transgenic mice containing a nucleic acid construct comprising a loxP-flanked neomycin resistance gene and the β-gal gene disrupting the RNA polymerase II locus (P2Bc . . .)" (emphasis added, see Office Action, Paper No. 25, page 3 line 19 through page 4, line 2). This is incorrect.

Instead, P2Bc (Pol II, β-gal, conditional target) was created via homologous recombination in ES cells containing the ProCre transgene (recombinase) plus the target nucleic

acid flanked by the loxP sites (target). Thus, ES cells containing both the Cre recombinase and the target for Cre-mediated recombination were then used to develop male mice. Upon development through spermatogenesis, the Cre-mediated recombination of these loxP sites results in deletion of the intervening sequence, resulting in the recombination event and generating the P2Br allele (Pol II, β-gal, recombined). Therefore, Example 3 clearly teaches ES cells containing ProCre and the second target sequence P2Bc, and isolating and manipulating ES cells from ProCre transgenic mice.

Based on the incorrect interpretation of the Example 3, the Examiner erroneously concludes that the "specification does not teach making ES cells with ProCre and P2Bc or isolating ES cells from ProCre/P2Bc transgenic mice" (see Office Action, Paper No. 25, at page 4, lines 6-7). The statement that "ProCre males bred with P2Bc females did not result in recombination" (emphasis added, see Office Action, Paper No 25, at page 4, lines 7-8) is exactly as expected, since only spermatogenesis would activate the recombinase and create the recombinant P2Br allele.

In fact, the Examiner directly contradicts his own statements made with respect to Example 3 (discussed in the previous paragraph) when discussing Example 5. Example 5 clearly shows that ProCre ES cell clones could be obtained from male mice containing homologously recombined recombinase. The Example also clearly shows that these ProCre ES cells could then be transfected with a second construct containing a target gene flanked by recombinase recognition sites, causing a Cre mediated excision event. The Examiner properly interprets portions of Example 5, when he acknowledges that the "specification does teach isolating male ES cell lines from the ProCre mice and transfecting them with a vector comprising a selectable marker flanked by two loxP sites" and that "transfected ES cells recombined such that the selectable marker was removed . . ." (see Office Action, Paper No. 25, at page 6, line 20 through page 7, line 3). Therefore, claims directed to ES cells containing two constructs (i.e., claims 13-15, 26, 28-32, 34-43 and 46-48) are clearly enabled by the specification as filed.

Furthermore, the working examples provide ample support for the claims as follows.

Claims 28-31 are directed to methods for excision of the transcriptionally active selectable marker from ES cells containing a germline-specific promoter associated with a recombinase coding sequence plus a selectable marker flanked by two recombinase recombination target sites. Appellant submits that the Examiner has in fact acknowledged enablement of these claims (see Office Action, Paper No. 25, at page 6, line 20 through page 7, line 3). The ES cells of the Example 3 contain Cre (the germline-specific recombinase) and the β-gal gene (the selectable marker) flanked by loxP (the target sites). When these ES cells are passaged through gametogenesis, the selectable marker is excised by the recombinase. The Examiner acknowledges that male ES cells from ProCre mice are transfected with a selectable marker which is later removed in Example 5 (see Office Action, Paper No. 25, at page 6, line 20 through page 7, line 3). Therefore, claims 28-31 are clearly enabled by the specification as filed.

Claims 32, 34-42 and 46-48 are directed to various methods for the production of recombinant alleles in a transgenic animal by introducing a nucleic acid fragment flanked by at least two recombinase recombination target sites into ES cells containing a germline-specific promoter associated with a recombinase coding sequence and obtaining transgenic progeny having a recombinant allele therein. Appellant submits that the Examiner has in fact acknowledged enablement of these claims (see Office Action, Paper No. 25, at page 6, line 20 through page 7, line 3. Example 3 produces P2Br progeny (containing the recombinant allele) from ES cells containing the β-gal gene (the nucleic acid fragment) flanked by loxP (the target sites) and ProCre (the germline-specific recombinase). The Examiner acknowledges that male ES cells from ProCre mice are transfected with a selectable marker, thereby creating the ES cells and resultant transgenic mice with the two constructs as claimed, in Example 5 (see Office Action, Paper No. 25, at page 6, line 20 through page 7, line 3). Therefore, claims 32, 34-42 and 46-48 are clearly enabled by the specification as filed.

As to the separate patentability of these method claims, they can be subdivided as follows. Claims 32 and 34, which stand or fall together, embrace the production of recombinant alleles in an animal using two nucleic acid constructs that cause a recombination event. Claim

46, which stands or falls alone, covers such a method in rodents, a narrowing subset of non-human animals. Claim 47, which stands or falls alone, covers such a method in mice, a narrowing subset of rodents. Claims 35-39, which stand or fall together, embrace the production of recombinant alleles in rodents using two nucleic acid constructs that do not cause a recombination event. Claim 48, which stands or falls alone, covers such a method in mice, a narrowing subset of rodents. Claims 40-42, which stand or fall together, embrace the production of recombinant alleles using only one nucleic acid construct.

Claim 43 is directed to a method for the conditional assembly of functional genes. Appellant submits that one of skill in the art would be able to apply the teachings of the invention in light of the disclosed teachings in the art, for example, US Patent No. 5,654,182 (see, for example, specification at page 14, lines 11-30) for this use of ES cells. Therefore, claim 43, which stands or falls alone as a distinct method, is enabled by the specification as filed.

Claim 44 is directed to a method for the generation of a recombinant non-human animal by combining a nucleic acid construct containing a germline-specific promoter operatively associated with a recombinase coding sequence with ES cells, and allowing the ES cells to grow and become an embryo in a host female to produce a recombinant by operation of the recombinase at gametogenesis. Appellant submits that the Examiner has in fact acknowledged enablement of this claim as well (see Office Action, Paper No. 25, at page 6, line 20 through page 7, line 3). Example 2 produces ProCre transgenic mice by developing ES cells containing ProCre (the germline-specific recombinase). The Examiner acknowledges that ProCre transgenic mice created with a germline-specific promoter and a recombinase are used in Example 5 (see Office Action, Paper No. 25, at page 6, line 20 through page 7, line 3). Therefore, claim 44, which stands or falls alone as a distinct method, is enabled by the specification as filed.

Appellant further disagrees with the Examiner's assertion that the "specification does not provide a use for ES cells or mice expressing a marker gene" (emphasis added, see Office Action, Paper No. 25, at page 7, lines 5-6). This statement is unreasonable, as it is clear that the

marker gene is simply a convenient tool for generating recombinant animals of interest. The utility of a transgenic animal with germline-specific recombination of a gene of interest is self-evident and well-established to one of skill in the art.

In summary, the specification "discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim", for each of the methods claimed. Therefore, the enablement requirement of 35 U.S.C. § 112 is satisfied (*In re Fisher*, 166 USPQ 18, 24 (CCPA 1970); MPEP § 2164.01(b)). Accordingly, one of skill in the art would not have to undertake undue experimentation to practice the invention as described in claims 28-32, 34-42, 44 and 46-48.

2. 35 U.S.C. §112, second paragraph Rejection of Claims 12, 28-32, 34-44 and 46-51

The rejection of claims 12, 28-32, 34-44 and 46-51 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite is respectfully submitted to be in error for the following reasons. Indeed, this rejection should be reversed because the Examiner has failed to interpret the claims in light of the specification as would one of ordinary skill in the art.

a. The *prima facie* case

The Examiner has the burden of providing reasons why the claim terminology is allegedly indefinite to one of skill in the art. The claims must be read as they would be interpreted by one possessing the ordinary level of skill in the pertinent art, in light of the specification and of teachings of the prior art. "If the claims, read in the light of the specification, *reasonably apprise those skilled in the art* both of the utilization and scope on the invention . . .", the claims are definite as required by 35 U.S.C. § 112, second paragraph (emphasis added, *North Am. Vaccine, Inc. v. American Cyanamid Co.*, 28 USPQ 2d 1333, 1339 (Fed. Cir. 1993)).

The Examiner has not considered both the content of the specification and the interpretation of the claims by one of skill in the art in formulating the rejections of record.

Instead, the Examiner has asserted a lack of clarity by offering alternative interpretations of the claims that are not consistent with the specification as considered by one of skill in the art. Therefore, the Examiner has failed to establish a *prima facie* case of indefiniteness.

b. The phrase "passaging the genome derived from said embryonic stem cells through gametogenesis" is definite to one of skill in the art in light of the specification

Appellant respectfully disagrees with the rejection of claims 28-32 and 34-39 as allegedly being indefinite because the phrase "passaging the genome derived from said embryonic stem cells through gametogenesis" is allegedly unclear. Appellant submits that the phrase is clear to one of skill in the art, taking into consideration the teachings of the specification as a whole.

The crux of the invention is that a germline-specific promoter, operatively associated with a recombinase coding sequence, can be used to "switch on" the transcription of this recombinase only in germline cells. Thus, the production of the recombinase can be controlled; and the resultant recombinase can be used with additional constructs providing target nucleic acid sequences flanked by target recombinase recombination target sites to achieve controlled recombination events in germline cells only (see, for example, specification at page 4, lines 12-17). The constructs are introduced into invention embryonic stem cells, where the recombinase is not expressed by the stem cells prior to developmental events leading to gametogenesis (see, for example, specification at page 15, lines 16-25).

One of skill in the art would immediately recognize that the only way to generate the desired recombinase would be to passage the ES cells through gametogenesis, i.e., to develop an embryo from the ES cells through the point at which either spermatogenesis or oogenesis occurs in the development of the embryo. The specification explicitly teaches the creation from invention ES cells of early embryos, and the introduction of such early embryos into intermediate hosts or surrogate females to be developed through gametogenesis (see, for example, page 9, lines 8-23; and Examples 2, 3 and 5).

In an effort to manufacture a lack of clarity in the subject phrase, the Examiner asserts that "[i]t is unclear if applicants intend to claim obtaining an animal from the ES cells and

breeding the animal such that the transgene is present in the gametes or whether the transgene is present until gametogenesis, spermatogenesis or oogenesis occurs" (see Office Action, Paper No. 25, at page 9, lines 1-3). Appellant respectfully submits that the latter interpretation is an impossibility, and that one of skill in the art would clearly understand the claims to mean development of the ES cells through the point of gametogenesis.

The methods of claims 28-32 and 34-39 all begin with ES cells, in which no recombinase is expressed as noted above. In order to achieve the desired event recited in the claims, i.e. excision or production of recombinant allele, the recombinase must be produced by the expression of the recombinase coding sequence. Transcription of the recombinase from the germline-specific promoter can only begin at gametogenesis. Clearly, the recombinase required to affect the recombination event must be generated when ES cells are developed into germline cells. Thus, the passaging step is required to achieve a recombination event mediated by the recombinase operatively linked to a germline-specific promoter, because no recombination event, and no transgene is ever possible prior to passaging the ES cells through gametogenesis where recombinase can be expressed. Therefore, the Examiner's example of the transgene being present until gametogenesis occurs is not consistent with the invention methods; and the only remaining possibility is that the ES cells are passaged through developmental stages, i.e. gametogenesis, where germline cells are generated.

Accordingly, the phrase "passaging the genome derived from said embryonic stem cells through gametogenesis" is respectfully submitted to be clear to one of skill in the art in light of the specification. Therefore, claims 28-32 and 34-39 cannot be indefinite based on the usage of this phrase.

c. The phrase "introducing a nucleic acid fragment" is definite to one of skill in the art in light of the specification

Appellant respectfully disagrees with the rejection of claims 12, 26, 32, 34-39 and 43 as allegedly being indefinite because the "introducing" step and the phrase "introducing a nucleic acid fragment" are allegedly unclear. Appellant submits that these terms are clear to one of skill in the art, when taking into consideration the teachings of the specification as a whole.

In an effort to manufacture a lack of clarity in the subject terms, the Examiner asserts that "[i]t is unclear if the nucleic acid fragment introduced in claims 32 and 34-39 is the nucleic acid construct in the ES cells referred to in the parent claims or if it refers to a second construct that is introduced into the cell" (see Office Action, Paper No. 25, at page 9, lines 6-8). Appellant respectfully submits that the Examiner's second suggestion with a second construct is clearly consistent with the claims. The Examiner's first suggestion that only one fragment is introduced in claims 32 and 34-39 is not consistent with the specification, and would never be considered by one of skill in the art.

Claim 12 includes a nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence. Claims 32, 34 and 43 are dependent on claim 12, and introduces further nucleic acid fragment(s) flanked by recombinase recombination target sites. It is clear to one of skill in the art that two distinct nucleic acid fragments are introduced into the ES cells, the first encoding the recombinase, and the second providing a recombination target for the recombinase. There can be no other interpretation by one of skill in the art in light of the specification's clearly articulated goal of achieving a recombination event mediated by the recombinase in germline cells as noted above.

Claim 26 similarly includes a germline-specific promoter operatively associated with a recombinase coding sequence; and a selectable marker flanked by recombinase recombination target sites. Claims 35-39, dependent on claim 26, are directed to methods for the production of recombinant alleles by introducing a nucleic acid fragment flanked by at least two recombination target sites. Again, it is clear to one of skill in the art that the claims require distinct nucleic acid fragments to be introduced into the ES cells: the first fragment encodes the recombinase, the second provides a selectable marker, and the third provides a recombination target for the recombinase to create the recombinant allele. There can be no other interpretation by one of skill in the art in light of the specification's clearly articulated goal of achieving a recombination event mediated by the recombinase in germline cells as noted above.

Accordingly, the "introducing" step and the phrase "introducing a nucleic acid fragment" are clear to one of skill in the art in light of the specification. Therefore, claims 12, 26, 32, 34-39 and 43 cannot be indefinite based on the usage of these terms.

d. The phrase "thereby producing a DNA which encodes a functional gene" is definite to one of skill in the art in light of the specification

Appellant respectfully disagrees with the rejection of claim 43 as allegedly being indefinite because the body of the claim allegedly does not reflect the preamble of the claim. Appellant submits that the subject phrase is clear to one of skill in the art, when taking into consideration the teachings of the specification as a whole.

Appellant respectfully disagrees with the Examiner's assertion that "[i]t is unclear whether the 'DNA' produced as newly amended correlates with the inactive gene segments or some other DNA present in the cell" (see Office Action, Paper No. 25, at page 9, lines 10-12). Appellant submits that the phrase "thereby producing a DNA which encodes a functional gene of interest" (emphasis added) clearly reflects the preamble of the claim "a method for the conditional assembly of functional gene(s)".

Claim 43 requires the ES cells of claim 12, containing a germline-specific promoter operatively associated with a recombinase coding sequence. The method contemplates introduction of individual inactive gene segments in these ES cells, wherein each segment contains at least one recombinase recombination target site. Upon passage of the genome derived from these modified ES cells through gametogenesis, the recombinase is produced, and the individual inactive gene segments are recombined to produce a DNA that encodes a functional gene. One of skill in the art would immediately recognize that this DNA is simply an assembly of the individual inactive gene segments by recombination into a functional gene.

Accordingly, the recited method steps clearly accomplish the goal set forth in the preamble of the claim. Therefore, claim 43 is definite and clear to one of skill in the art in light of the specification.

In re Application of: O'Gorman and Wahl
Application No.: 08/919,501
Filing Date: August 28, 1997

PATENT
Attorney Docket No.: SALK2190
(088802-5001)

X. Conclusion

For the foregoing reasons, the Examiner's rejections of claims 12-15, 18-24, 26, 28-32, 34-44 and 46-51 are respectfully submitted to be in error. Accordingly, reversal and allowance of all claims are respectfully requested.

Respectfully submitted,

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Enclosures: Appendices A and B

In re Application of: O'Gorman and Wahl
Application No.: 08/919,501
Filing Date: August 28, 1997

PATENT
Attorney Docket No.: SALK2190
(088802-5001)

Appendix A: Claims Involved in the Appeal

12. (Twice amended) Non-human mammalian embryonic stem cells containing a nucleic acid construct comprising a mammalian germline-specific promoter operatively associated with a recombinase coding sequence, wherein the nucleic acid construct is in the genome of the stem cells and wherein the recombinase is not expressed in the stem cells in cell culture.

13. (Twice amended) Embryonic stem cells according to claim 12 wherein the genome thereof further comprises a transcriptionally active selectable marker flanked by two recombinase recombination target sites.

14. (Amended) Embryonic stem cells according to claim 13 wherein the recombinase encoded by the recombinase coding sequence is selective for the recombination target sites flanking said selectable marker.

15. (Amended) Embryonic stem cells according to claim 13 further comprising one or more of:

a nucleic acid fragment flanked by two recombinase recombination target sites, wherein said recombination target sites are different than the recombination target sites which flank said selectable marker,

a nucleic acid construct comprising an inducible promoter operatively associated with a recombinase coding sequence, or

a nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

18. (Amended) Embryonic stem cells according to claim 12 wherein said recombinase coding sequence encodes Cre recombinase.

19. (Amended) Embryonic stem cells according to claim 18 wherein said construct is ProCre, comprising the protamine 1 gene promoter operatively associated with Cre recombinase.

20. (Amended) Embryonic stem cells according to claim 12 wherein said recombinase coding sequence encodes FLP recombinase.

21. (Amended) Embryonic stem cells according to claim 20 wherein said construct is ProFLP, comprising the protamine 1 gene promoter operatively associated with FLP recombinase.

22. (Amended) Embryonic stem cells according to claim 12 wherein said recombinase coding sequence encodes the R gene product of *Zygosaccharomyces*.

23. (Amended) Embryonic stem cells according to claim 22 wherein said construct is ProR, comprising the protamine 1 gene promoter operatively associated with the R gene product of *Zygosaccharomyces*.

24. (Twice amended) Embryonic stem cells according to claim 12 further comprising an inducible promoter operatively associated with a recombinase coding sequence and a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells.

26. (Thrice amended) Non-human mammalian embryonic stem cells comprising a germline-specific promoter operatively associated with a recombinase coding sequence and a transcriptionally active selectable marker flanked by two recombinase recombination target sites in the genome of the stem cells.

28. (Thrice amended) A method for excision of the transcriptionally active selectable marker from the embryonic stem cells of claim 26, said method comprising:

passaging the genome derived from said embryonic stem cells through gametogenesis, wherein said passaging causes excision of the transcriptionally active selectable marker.

29. A method according to claim 28 wherein said genome is passaged through spermatogenesis.

30. A method according to claim 28 wherein said genome is passaged through oogenesis.

31. (Amended) A method according to claim 28 wherein said embryonic stem cells further comprise one or more of:

a nucleic acid fragment flanked by two recombinase recombination target sites, wherein said recombination target sites are different than the recombination target sites which flank said selectable marker,

a nucleic acid construct comprising an inducible promoter operatively associated with a recombinase coding sequence, or

a nucleic acid construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

32. (Thrice amended) A method for the production of recombinant alleles in a transgenic non-human animal, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombinase recombination target sites into mammalian embryonic stem cells of claim 12; and

passaging the genome derived from said embryonic stem cells through gametogenesis to obtain a transformed gamete; and

obtaining progeny from the transformed gamete, thereby producing a transgenic non-human animal having a recombinant allele therein.

34. A method according to claim 32 wherein said nucleic acid fragment is introduced by homologous recombination, random insertion, retroviral insertion, or site specific-mediated recombination.

35. (Thrice amended) A method for the production of recombinant alleles in a rodent, said method comprising:

introducing a nucleic acid fragment flanked by at least two recombination target sites into embryonic stem cells of claim 26, wherein said cells are rodent cells,

passaging the genome derived from said embryonic stem cells through gametogenesis without causing recombination of the recombination target sites,

producing offspring resulting from crossing the genome of a gamete produced by the gametogenesis with the genome of a wild type rodent,

whereby the nucleic acid fragment is inserted into the genome of the offspring and produces the recombinant allele therein.

36. (Amended) A method according to claim 35 wherein said embryonic stem cells further comprise a second nucleic acid construct selected from the group consisting of a construct comprising an inducible promoter operatively associated with a recombinase coding sequence and a construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

37. A method according to claim 36 wherein the recombinase encoded by said second construct is expressed in response to inducing conditions.

38. A method according to claim 36 wherein the recombinase encoded by said second construct is expressed in a tissue selective manner.

39. (Amended) A method according to claim 35 wherein the recombination target sites flanking said nucleic acid fragment are recognized by a recombinase which is expressed under the control of an inducible promoter or a tissue specific promoter.

40. (Thrice amended) A method for the production of recombinant alleles, said method comprising:

introducing at least one nucleic acid construct into the genome of mammalian embryonic stem cells,

wherein said at least one nucleic acid construct comprises a germline-specific promoter operatively associated with a recombinase coding sequence, a nucleic acid fragment flanked by a first pair of recombination target sites and a selectable marker flanked by a second pair of recombination target sites,

passaging the genome derived from embryonic stem cells selected for expression of the marker through gametogenesis to obtain a transformed gamete; and

crossing the genome of the transformed gamete with the genome of a wild type animal, thereby obtaining first generation progeny wherein the marker is excised in the germline.

41. (Twice amended) A method according to claim 40 wherein said first pair of recombination target sites is recognized by a recombinase which is expressed under the control of a germline-specific promoter and said second pair of recombination target sites is recognized by a recombinase which is expressed under the control of an inducible promoter or a tissue specific promoter.

42. (Amended) A method according to claim 40 wherein said embryonic stem cells further comprise a second nucleic acid construct selected from the group consisting of a construct comprising an inducible promoter operatively associated with a recombinase coding sequence and a construct comprising a tissue-specific promoter operatively associated with a recombinase coding sequence.

43. (Thrice amended) A method for the conditional assembly of functional gene(s) for expression in eukaryotic cells by recombination of individual inactive gene segments from one or more gene(s) of interest,

wherein each of said segments contains at least one recombinase recombination target site, and wherein at least one of said segments contains at least two recombinase recombination target sites,

said method comprising:

introducing said individual inactive gene segments into a mammalian embryonic stem cell of claim 12, wherein recombinase is expressed, thereby producing a DNA which encodes a functional gene of interest, the expression product of which is biologically active, upon passage of the genome derived from said embryonic stem cells through gametogenesis.

44. (Thrice amended) A method for the generation of recombinant non-human animal, said method comprising:

combining a nucleic acid construct comprising a germline-specific promoter operatively associated with a recombinase coding sequence with host pluripotential ES cells derived from early preimplantation embryos,

introducing these embryos into a host female, and

allowing the derived embryos to come to term such that a recombinant non-human animal is thereby produced by operation of the recombinase upon passage of the genome derived from the embryonic stem cell through gametogenesis.

46. The method according to claim 32 wherein the non-human animal is a rodent.

47. The method according to claim 46 wherein the rodent is a mouse.

48. The method according to claim 35 wherein the rodent is a mouse.

49. (Amended) The cells according to claim 12 wherein the non-human mammalian embryonic stem cell is a rodent cell.

50. (Amended) The cells according to claim 49 wherein the rodent is a mouse.

51. (Amended) The cells according to claim 12 wherein the non-human mammalian embryonic stem cell is a livestock stem cell.

APPENDIX B

1: Proc Natl Acad Sci U S A 1987 Aug;84(15):5316-9 Related Articles, Protein, Books

Spermatid-specific expression of protamine 1 in transgenic mice.

Peschon JJ, Behringer RR, Brinster RL, Palmiter RD.

Protamines are abundant basic proteins involved in the condensation of sperm chromatin. In the mouse, protamine genes are transcribed postmeiotically in round spermatids. We have cloned and sequenced the mouse protamine 1 gene. Ten lines of transgenic mice harboring marked protamine 1 sequences were generated by microinjection of fertilized eggs. Transcription of the transgene is restricted to round spermatids and in several cases exceeds that of the endogenous gene. The cis-acting sequences required for tissue-specific protamine expression reside on a 2.4-kilobase restriction fragment. Prospects for using transgenic mice to address fundamental questions of male germ-cell development are discussed.

PMID: 3037541 [PubMed - indexed for MEDLINE]

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1: Ann N Y Acad Sci 1989;564:186-97

Related Articles, Books

Expression of mouse protamine 1 genes in transgenic mice.

Peschon JJ, Behringer RR, Palmiter RD, Brinster RL.

Department of Biochemistry, University of Washington, Seattle 98195.

Mouse protamine genes are expressed exclusively in spermatids. Mouse protamine 1 (mP1) transcriptional regulatory elements can target the expression of either marked mP1 transgenes or mP1 chimeric genes to spermatids in transgenic mice. Sequences between -40 and -465 bp relative to the transcription start site are required for expression in spermatids, whereas sequences 3' of the point of translation initiation are dispensable. mP1 transcriptional regulatory sequences were used to direct the expression of a toxic gene product to spermatids. The phenotypic consequences of toxin expression in spermatids are described.

PMID: 2774416 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A 1993 Jun 1;90(11):5071-5

Analysis of the mouse protamine 1 promoter in transgenic mice.

Zambrowicz BP, Harendza CJ, Zimmermann JW, Brinster RL, Palmiter RD.

Howard Hughes Medical Institute, University of Washington, Seattle 98195.

Protamines are small arginine-rich proteins that package DNA in spermatozoa. The mouse protamine 1 (Prm-1) gene is transcribed exclusively in post-meiotic spermatids. To identify elements in the Prm-1 promoter required for spermatid-specific transcription, we generated transgenic mice by microinjection of transgenes containing Prm-1 5' flanking sequences with 5' truncations or internal deletions of conserved sequences linked to a marked Prm-1 gene. We also tested Prm-1 promoter regions with a heterologous human growth hormone reporter gene. We conclude that a 113-bp region can direct spermatid-specific transcription and we have defined sequences within this region that are essential for proper function. These results will facilitate the isolation and characterization of transcription factors essential for post-meiotic gene expression.

PMID: 8389466 [PubMed - indexed for MEDLINE]